

**1251-Pos Board B161****Direct Calorimetric Determination of a Complete Polyproline II (pII) Propensity Scale Reveals PII Enhancement in Intrinsically Disordered Proteins**

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Circular dichroism spectropolarimetry experiments and other studies have suggested that the denatured states of polypeptides may comprise a smaller conformational space than previously thought, and that the polyproline II (PII) helix is one of the conformations that is highly populated in the denatured state. Src-homology 3 (SH3) is a modular domain that recognizes and binds peptide in the PII conformation, such as SosY, with high specificity. Our experimental strategy is to use isothermal titration calorimetry (ITC) to monitor the binding of SH3 to engineered SosY peptides, enabling the direct detection of ligand population that is in the PII conformation. Importantly, our model system allows direct access to free energy information concerning the context dependent PII propensity of amino acids at specific sites in the SosY peptide. Binding isotherms have been measured and quantitative estimates made for the PII propensity all twenty amino acids, developing a complete PII scale. Correlations of the calorimetrically determined scale to the GenomeNet database of amino acid physico-chemical property and secondary structure propensity scales revealed no significant correlations. An algorithm is developed to calculate the average PII propensities of different sequences, revealing that intrinsically disordered proteins have an enhancement of PII compared to sequences that fold. Upon random shuffling, the high PII regions within intrinsically disordered segments are lost, demonstrating local enrichment of high PII bias within these sequences.

**1252-Pos Board B162****MD Simulations Highlight the Contrast in Dynamics of Intrinsically Disordered Proteins When Compared with Folded Proteins**

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Intrinsically Disordered Proteins (IDPs) lack a stable three-dimensional structure in substantial regions or throughout their sequence and exist as highly dynamic conformational ensembles. However, IDPs perform essential biological functions like regulation or signalling where their unique structural plasticity plays a crucial role. They comprise nearly 30% of proteins in the eukaryotic genome. Many IDPs are found to be associated with human diseases like cancer, cardiovascular disease, amyloidoses, neurodegenerative diseases and diabetes. Regardless of their abundance and importance, structural characterization of IDPs remains a challenge that is poorly addressed. It is difficult to characterize the disordered proteins because of their heterogeneity and rapid inter-conversion of conformers leading to practical challenges. Here, we attempted to unravel the characteristic features of intrinsically disordered proteins using Molecular Dynamics simulation as this method will yield a large ensemble of diverse structures and provide tools to analyze structure and associated dynamics. In this study, we have used multiple MD simulations to compare the conformational dynamics originating from two ordered proteins (PDB codes: 1BGF, 1MUN), one partially ordered protein (2HDL), and four disordered proteins (2SOB, 1LXL, 1VZS and 1JH3). All the seven simulations (10 ns each) were performed using ff99SB Amber force field. Analysis of the trajectories arising from these simulations in terms of parameters such as, RMSD, solvent accessible surface area, secondary structure analysis and conformational entropy provide valuable insights on the structure and dynamics of disordered regions/proteins in comparison with ordered regions/proteins.

**1253-Pos Board B163****Structured Functional Domains of Myelin Basic Protein: Cross-Talk Between Actin Polymerization and  $\text{Ca}^{2+}$ -Dependent Calmodulin Interaction**

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The 18.5 kDa myelin basic protein (MBP), the most abundant isoform in human adult myelin, is a multifunctional, intrinsically disordered protein that maintains compact assembly of the sheath. A hydrophobic moment analysis of MBP's amino acid sequence reveals three regions with high propensity to form strongly amphipathic  $\alpha$ -helices. These regions, located in the central, N- and C-terminal parts of the protein, have been shown to play a role in the interactions of MBP with other partners, such as SH3-domain binding proteins, actin,  $\text{Ca}^{2+}$ -activated calmodulin ( $\text{Ca}^{2+}$ -CaM), and with myelin-mimetic membrane bilayers. Here, we have further characterized the structure-function relationship of these three domains. We constructed three recombinant peptides derived from the 18.5 kDa murine MBP: A22-K56, S72-S107, and S133-S159 (denoted  $\alpha$ 1,  $\alpha$ 2, and  $\alpha$ 3, respectively). We used a variety of biophysical methods (circular dichroism spectroscopy, solid-state NMR (ssNMR) spectroscopy, isothermal titration calorimetry, electron microscopy, and fluorimetry) to characterize the interactions of these peptides with actin and calmodulin. Our results show that all three peptides can adopt  $\alpha$ -helical structure inherently. Although both  $\alpha$ 1 and  $\alpha$ 3 peptides showed strong

binding with  $\text{Ca}^{2+}$ -CaM, only  $\alpha$ 1 exhibited actin polymerization and bundling activity. Calmodulin depolymerized actin that was polymerized by  $\alpha$ 1. Comparing fingerprints of Ala, Pro, and Ser (using ssNMR) in a reconstituted  $\alpha$ 1-actin complex, we showed that this peptide might adopt a better-defined structural state but still exhibits structural polymorphism. The results of this study proved that in addition to the primary calmodulin-binding site located in the C-terminal domain of MBP, there is another N-terminal binding domain for  $\text{Ca}^{2+}$ -CaM. This secondary binding domain appeared to be essential for CaM-induced actin depolymerization.

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**1254-Pos Board B164****Modes of SH3-Domain Interactions of 18.5 kDa Myelin Basic Protein *In Vitro* and in Oligodendrocytes**

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The developmentally-regulated myelin basic proteins (MBPs) play key roles in central nervous system (CNS) myelin formation by oligodendrocytes. They are highly positively-charged, intrinsically disordered, multifunctional proteins having several alternatively-spliced isoforms and combinatorial post-translational modifications. The most common 18.5 kDa MBP isoform contains a proline-rich region (murine sequence T92PRTTPPS99) which comprises a minimal SH3-ligand. We have previously shown that 18.5 kDa MBP binds to Fyn, a member of the Src family of tyrosine kinases involved in signaling pathways during CNS development. Here, we have produced an isotopically-labeled fragment of MBP which contains the proline-rich region, and are studying the mode of interaction between MBP and the SH3 domain of Fyn through solution NMR spectroscopy and isothermal titration calorimetry (ITC). We have constructed MBP variants pseudo-phosphorylated at T92 and T95, and with P93G and P96G substitutions to disrupt the conformation of the SH3-ligand. Using ITC, these mutant MBPs are shown to have a decreased affinity towards the SH3-domain of Fyn. Solution NMR spectroscopy shows altered chemical shift patterns for the different variants, confirming that this segment is indeed the SH3-target and that the association can be modulated by phosphorylation. Concomitant over-expression of complementary GFP-tagged MBP forms in cultured oligodendrocytes results in aberrant elongation of membrane processes, increased branching complexity, and in some cases, trafficking of MBP to the nucleus. These data as a whole indicate that MBP's SH3-ligand domain plays a key role in intracellular protein interactions and may be required for proper membrane elaboration in myelin.

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**1255-Pos Board B165****Intrinsically Disordered Islet Amyloid Polypeptide Is a Pathogenic Link Between Type-2 diabetes and Heart Disease**

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Islet amyloid polypeptide (IAPP) is an intrinsically disordered protein co-expressed and secreted with insulin by pancreatic  $\beta$ -cells. IAPP is produced at increased rates in patients with pre-diabetes, leading to proteotoxicity and extracellular amyloid deposition, a hallmark of type-2 diabetes. We found high IAPP levels in blood and failing hearts from pre-diabetic and diabetic humans, showing that IAPP accumulates in the heart and may contribute to heart dysfunction. Here, we tested the hypothesis that accumulation of IAPP in the heart alters Ca handling in cardiac myocytes, accelerating the occurrence of heart failure. We measured Ca transients in rats transgenic for human IAPP (HIP rats) and in wild-type RIP rats that bear only the non-diabetogenic IAPP isoform. Ca transient amplitude was significantly larger in cardiac myocytes from *pre-diabetic* HIP rats vs. age-matched control rats. In contrast, *pre-diabetic* RIP rats showed unaltered Ca transients. We also found increased Ca transients in myocytes incubated with exogenous human IAPP but not with rodent IAPP. Increased cellular Ca load is involved in hypertrophic signaling and pathological remodeling of the heart. We measured the level of brain natriuretic peptide (BNP), a molecular marker of hypertrophy, in heart protein homogenates from HIP rats using western blots. BNP expression was already elevated (by  $70 \pm 21\%$ ) in hearts from pre-diabetic rats vs. age-matched control littermates and further increased with diabetes development. These data show that cardiac Ca dysregulation and hypertrophy correlates with toxic deposition of IAPP in the heart. In conclusion, our data suggest that accumulation of IAPP oligomers contribute significantly to the pathogenesis of diabetic heart failure. IAPP represents an effective target for diagnostic purposes and therapeutic strategies.